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(71) Applicant *(for all designated States except US)*: ABERDEEN UNIVERSITY [GB/GB]; Auris Business Centre, 23 St. Machar Drive, Aberdeen AB2 1RY (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: MELVIN, William, Thomas [GB/GB]; 5 Deeside Park, Aberdeen AB17 PQ (GB). DUNCAN, Margaret, Ellen [GB/GB]; 19 Hillview Road, Banchory AB31 4EG (GB). FOTHERGILL, John, Edward [GB/GB]; The Granary, Shawhead, Stonehaven AB3 2JY (GB). MURRAY, Graeme, Ian [GB/GB]; 29 Woodhill Road, Aberdeen AB15 55U (GB).

(74) Agents: STEBBING, Peter, John, Hunter et al.; Ablett & Stebbing, 45 Lancaster Mews, Lancaster Gate, London W2 3QQ (GB).

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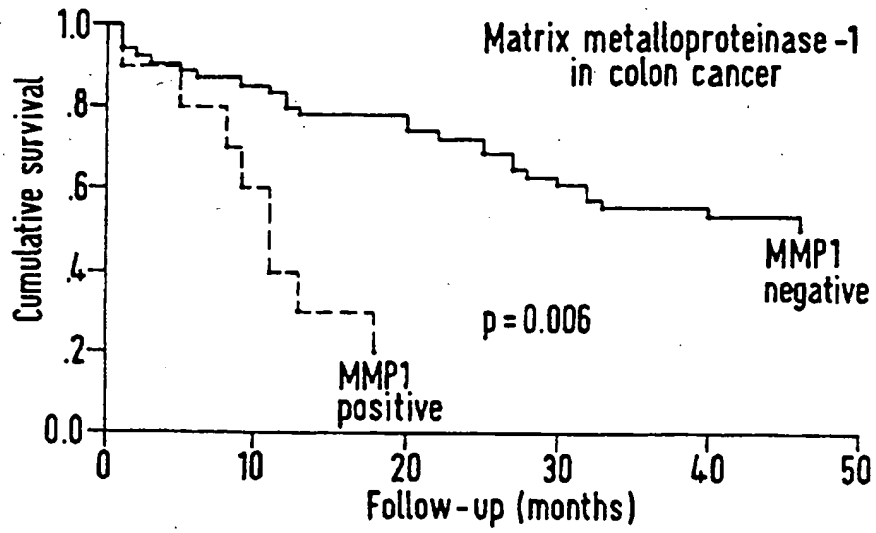

(57) Abstract

The invention provides a method for the *in vitro* prognostic evaluation of colorectal, oesophageal or ovarian cancer status from a sample with a monoclonal or polyclonal antibody specific respectively for a selected matrix metalloproteinase and screening for a positive response. The selected matrix metalloproteinase may be a collagenase or a gelatinase, particularly an MMP-1 or an MMP-9.

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PROGNOSTIC AND THERAPEUTIC SYSTEM FOR CANCER

The present invention relates to a prognostic and therapeutic system and further to the production of antipeptide monoclonal antibodies to an activated matrix metalloproteinase-1 and -9 proteins and to a therapeutic or diagnostic system incorporating such antibodies.

Colon, prostate, ovarian and oesophageal cancers are among the commonest malignant tumours with a relatively poor prognosis. The outcome of such cancers depends on the extent of both local and particularly metastatic tumour spread. Tumour invasion and metastases result from a multi-step process, one key step of which is degradation of the extracelluar matrix by proteolytic enzymes. Initial tumour invasion depends upon the destruction of the basement membrane whereas further spread of tumour cells requires digestion of the interstitial connective tissue. The activity of matrix metalloproteinase (MMP) group of proteolytic enzymes is normally strictly regulated by inhibitors such as TIMPs but in cancerous conditions they play a major role of degrading extracelluar matrix and it has a key role in facilitating tumour invasion and spread. For example, MMP-1 interstitial collagenous is a main MMP capable of degrading interstitial connective tissue. The applicants have studied the expression of MMP-1, MMP-2 and MMP-9 in colon, ovarian and oesophageal cancers and determined their prognostic significance.

The extracelluar matrix consists of two components, basement membrane and interstitial connective tissue both of which can be digested by a matrix metalloproteinase. Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes which share specific properties. These enzymes act to cleave components of the extracelluar matrix, with substrate specificity varying between metalloproteinases. All of the members of the metalloproteinase family require a metal atom bound at the active site to function. They show optimal

activity at neutral pH. Metalloproteinases are synthesized as inactive pro-enzymes which are activated by cleavage of a pro-peptide.

Matrix metalloproteinases are products of related genes and show much homology of their genetic sequences between members of the enzyme family. Thus far many distinct members of the metalloproteinase family have been identified, of which the following are examples.

Table 1.

The Properties of Matrix Metalloproteinases

	MMP	No.
Collagenases	interstitial collagenous	MMP-1
	neutrophil collagenous	MMP-8
Gelatinases	gelatinase A (72kDa)	MMP-2
	gelatinase B (92kDa)	MMP-9
Stromelysins	stromelysin1	MMP-3
	stromelysin2	MMP-10
	stromelysin3	MMP-11
Matrilysin (pump 1)		MMP-7
Metalloelastase		MMP-12

From The New Scientist (Coghlan) 26 March 1994, it is known that tumour spread is assisted by some MMPs which destroy the connective tissue between cells and organs allowing tumour cells to break out of their original site and enter new areas.

Whilst is has therefore been generally understood that matrix metalloproteinases are involved in the spread of cancers and it has also been recognised that this might correlate with the status of the cancers (see for example Jos et al., Int J Cancer, vol 45 (1990) p1071-1078), there have been no indications that individual members of the matrix metalloproteinase family might correlate with the status of the cancers especially in relation to survival of the patient. The present invention is based on the surprising discovery that the production of individual members of the matrix metalloproteinase family are highly significant for the survival of patients with certain cancers and that other members of the matrix metalloproteinase family are not significant in relation to survival with the same cancers.

We have now found that matrix metalloproteinase expression of the collagenases is significant, particularly for cancers such as colorectal, prostate and oesophageal cancer and that gelatinases, particularly MMP-9, are important markers for ovarian cancer.

A synthetic inhibitor of MMPs under the name Marimastat is now undergoing clinical trials. This binds to the zinc atom at the active site inhibiting extracelluar matrix degradation and possibly tumour metastases.

The invention is thus predicated on the discovery that in tumours such as colorectal and ovarian cancers, MMP-1 and MMP-9 are found to be produced in the more aggressive forms of disease where the prognosis in terms of life expectancy is shortest. It is also predicated on the basis that this discovery gives rise to effective therapeutic targets.

According to the first feature of the present invention therefore there is provided a method for the in vitro prognosis evaluation of cancer status from a sample, which method comprises contacting said sample with a monoclonal or

polyclonal antibody specific for a selected matrix metalloproteinase such as a collagenase or a gelatinase, and screening for a positive response. The positive response may be indicated by an agglutination reaction or by a visualisable change such as a colour change or fluorescence e.g. immunostaining, or by a quantitative method such as the use of radiochemical methods or enzyme-linked antibody (Eliza) methods.

In a preferred form of the invention, the antibody is a monoclonal antibody which is specific for the collagenous MMP-1 or the gelatinase B; MMP-9.

The invention also provides an antipeptide monoclonal antibody which is reactive with amino acid residues 267 to 277 of the activated MMP-1 protein, or antipeptide antibodies reactive with amino acid residues 603 to 614 of the MMP-9 protein (Swiss prot database numbering, identification number C0G9).

The peptides may be synthesized with a cysteine residue included at the N-terminus to enable coupling to a carrier protein with for example MBS, (m-maleimidobenzoyl-N-hydroxysuccinimide ester) and then coupled to ovalbumin and bovine serum albumin. Polyclonal or monoclonal antibodies can then be produced in the usual way.

The antibodies of the present invention may also be used to produce therapeutic agents comprising antibodies reactive with the matrix metalloproteinases of the present invention, such antibodies being complexed with toxic, radioactive or other molecules capable of exerting an antitumour effect either directly or indirectly, (for example by stimulating a biological response or by converting a non-toxic molecule to a toxic molecule in the vicinity of the tumour). It is also possible that the action of MMPs in general and the collagenases and gelatinases in particular, can be modified for example by preventing activation of MMPs, by blocking

access of substrates or cofactors to catalytic sites on the enzyme, by altering enzyme conformation or by mimicking the peptide which is cleaved from the pro-enzyme, by reproducing the action of TIMPs, by blocking binding to the tumour cell surface or by inhibition of the MMP catalytic sites. It is therefore possible for therapeutic antibodies to have direct inhibitory effects of the activity of MMPs or that they can be used in conjunction with enzyme inhibitors to either introduce these inhibitors to the vicinity of the tumour or to potentiate the action of these inhibitors.

In a further aspect of the present invention there is provided a therapeutic agent comprising an antipeptide monoclonal antibody to a metalloproteinase, especially a collagenase or gelatinase B, bound to either an anti tumour agent or an MMP inhibitory agent. The metalloproteinase is preferably an MMP-1 or MMP-9. For MMP-1, the antibody preferably corresponds to amino acid residues 267 to 277 of the activated MMP-1 protein. For MMP-9, the antibody preferably corresponds to residues 603 to 614 of the MMP-9 protein. The antitumour agent may comprise the antibody complexed to a cytotoxic drug or agent or alternatively may indirectly trigger the release of an antitumour drug or agent in the vicinity of the cancer. Other therapeutic agent configurations are possible, for example where the antipeptide monoclonal antibody is complexed to a liposomal or other encapsulated preparation of an antitumour drug including a range of antitumour moieties including toxic chemicals, toxins, genes or even MMP enzyme inhibitors. The MMP-1 inhibitory agent may have the effect selected from preventing activation for example mimicking the peptide residues which are cleaved from a pro-enzyme, or by reproducing the action of a TIMP, or by blocking tumour cell surface binding or by inhibition of MMP catalytic sites.

The present invention illustrates the general principle that the presence of individual matrix metalloproteinases can be highly significant in the prognosis of certain cancers and it is apparent that there are likely to be other examples where matrix metalloproteinase production will relate to tumour prognosis. It will now be obvious to those skilled in the art that the present invention could therefore be applied in a number of ways following the identification of individual matrix metalloproteinases with high prognostic significance. In diagnostic applications, individual MMPs could be measured as an indicator of the prognosis of the cancer which might guide the clinician in choice of therapy for the individual patient. For clinical trials, MMPs could be used as markers to identify patients with good and bad prognosis prior to selection of patients for trials and MMPs could then be used as surrogate marker end-points as an alternative to patient survival to accelerate the trials. For therapy, specific MMP's could either be inhibited or targeted as a means for destroying or retarding tumour cells involved in the progression of the cancer. It will now be obvious to those skilled in the art that antibodies of the present invention could be used to direct cytotoxic agents or other molecules capable of exerting an antitumour effect either directly or indirectly (for example by stimulating a biological response or by converting a non-toxic molecule to a toxic molecule in the vicinity of the tumour) or to direct other agents such as imaging agents to the tumour cells since the metalloproteinase has been shown to be present at the tumour cell surface. Agents derived from the antibodies such as Fab^2 or Fab molecules produced by enzymatic digestion or molecules such as scFv or humanized antibody molecules produced by recombinant DNA methods may also be used for this purpose.

The invention will now be described by way of illustration only by reference to the following examples and the accompanying Figures 1 to 4. Figures 1 to 3 show in graphic form the production of MMP-1 and MMP-9 and survival of patients with colorectal, oesophageal and ovarian cancers respectively, and Figure 4 shows a plot of an assay to show antibodies specific for individual MMP's.

EXAMPLE 1 - Generation of Anti-MMP Antibodies

Selection of Peptide and Configuration to Barrier Protein

The region chosen for generation of a first anti-peptide monoclonal antibodies was SSFGFPRTVKH. This corresponds to amino acid residues 267-277 of the activated MMP-1 protein. Similarly anti-peptide monoclonal antibodies for MMP-2 and MMP-9 were produced, respectively corresponding to amino acid residues 557 to 569 and 603 to 614. Specificity of Anti MMP-1, MMP-2 and MMP-9 (Western Blots) are shown in Figure 4. The selected peptide were synthesised at the Krebs Institute, University of Sheffield, with a cysteine residue included at the N-terminus to enable coupling to carrier proteins by m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS). In order to confirm a correct synthesis, the peptide were analyzed by mass spectrometry and amino acid sequencing.

The peptides were coupled to hen ovalbumin and bovine serum albumin (BSA) (Fluka) by an adaption of the method described in Sambrook et al (1989). The conjugates were analyzed by mass spectroscopy to ensure successful coupling and their concentrations determined by amino acid analysis.

Immunisation of Mice and Generation of Hybridoma Lines and Clones

Female BALB/c mice were injected intraperitoneally with the peptide-ovalbumin conjugates in PBS. The first and second injections were 100 μg conjugate in Freund's complete and incomplete adjuvant, respectively, at an interval of 2 weeks. Three months later the mice were tested for antibody production by tail bleeds, and the best responding mouse received a third injection of 400 μg of conjugate with no adjuvant. Three days after the final injection, the mouse spleen was removed for the production of hybridomas by fusion of the spleen cells with mouse myeloma Ag8.653 cells by standard methods (Kohler and Milstein, 1975; Barnes et al 1987). Antibody-producing hybridomas were identified by ELISA and immunoblotting and cloned twice by limiting dilution. Clones were isotyped using an Isostrip kit (Boehringer Mannheim).

ELISA

Wells of 96-well microtitre plates (Titertek, Flow Laboratories) were coated with peptide-BSA conjugate at 7.2 μg/ml in 50 mM carbonate-bicarbonate buffer, pH 9.6, by incubation overnight at 4°C. The wells were washed once with PBS-Tween (PBS containing 0.05% Tween 20) and unoccupied non-specific protein-binding sites on the wells were then blocked by incubation with 1% BSA in 50 mM sodium carbonate/bicarbonate buffer, pH 9.6. The wells were washed 3 times with PBS-Tween and then incubated with samples to be tested for antibodies (undiluted cell culture supernatant or 1/100 and further doubling dilutions of mouse serum). The wells were washed 3 times as before and incubated with alkaline-phosphatase-conjugated goat anti-mouse IgG (F_c specific, Sigma cat. no. A2429), diluted 1/2500 in PBS-Tween containing 0.5% BSA. After 3 more washes, the alkaline phosphatase substrate p-nitrophenyl phosphate was added at

0.1% in 0.1 M glycine/NaOH buffer, pH 10.4, containing 1 mM $ZnCl_2$ and 1 mM $MgCl_2$. All of the above incubations were at 37°C for one hour and volumes per well were 50 µl except for blocking and washing where 200 µl were used. The plates were read at 405 nm against a reference of 492 nm in a Titertek Multiscan plate reader to measure the formation of p-nitrophenol.

SDS-PAGE and Western Blotting

SDS-PAGE was carried out on 10% gels by the method of Laemmli (1970), using a Hoefer Mini-Gel apparatus. Electroblotting from the gels to polyvinylidene difluoride (PVDF) membranes (Problott, Applied Biosystems) was carried out in a Sigma blotting tank for two hours at 250 mA constant current in a 10 mM 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS)/10% methanol, pH 11, by the method of Matsudaira (1987). Sections of membrane were either stained for protein with Amido black (0.1% in 40% methanol/1% acetic acid) or immunostained as described previously (Duncan et al 1992) using the same second antibody as in the ELISA at a dilution of 1/500. Natural human pro-MMP-1 and recombinant human pro-MMP-2, pro-MMP-3 and pro-MMP9 were gifts from British Biotechnology. Prestained molecular weight markers were obtained from Biorad.

Immunohistochemistry

Sections of tumour were immunostained with a monoclonal antibodies to MMP-1, MMP-2 and MMP-9 using an alkaline phosphatase anti-alkaline phosphatase method as previously described.

Formalin fixed wax embedded sections (4µm) of tissue were dewaxed in xylene, rehydrated in alcohol and then washed sequentially in cold water and 0.05M Tris-HCl (pH 7.6) containing 0.15M sodium chloride (TBS). Sections of tissue were incubated for one hour with the MMP antibodies. Sites

of antibody binding were demonstrated using an alkaline phosphatase anti-alkaline phosphatase (APAAP) technique. Rabbit anti-mouse immunoglobulin (1/100 containing 1% normal human serum, Dako) and monoclonal APAAP (1/100, Dako) were sequentially applied to the tissue sections for 30 minutes each. Between antibody applications the sections were washed with TBS to remove unbound antibody. Sites of bound alkaline phosphatase were identified using an enzyme substrate solution containing 3 mg bromo-chloro-indolyl phosphate (Sigma Chemical Co Ltd, Poole, Dorset), 10 mg nitro blue tetrazolium (Sigma), 6 mg sodium azide and 4 mg levamisole (Sigma) in 10 ml 0.05M Tris-HCl buffer (pH 9.0) containing 0.2% magnesium chloride. After incubating the sections for 30 minutes at room temperature, the reaction was stopped by washing the section sin cold tap water. The slides were then air-dried and mounted in glycerine jelly.

Sections were subjected to an antigen retrieval step by microwaving the sections for twenty minutes in 0.01M citrate buffer pH 6.0 prior to application of the primary antibody. The slides were examined using light microscopy by two observers in order to determine qualitatively the presence or absence of immunostaining, and its distribution. Individual tumours were classified as positive if five percent or more of the tumour cells were positive. The follow-up data was obtained after determination of MMP immunoreactivity in the tumours.

Cumulative patient survival was assessed by the method of Kaplan-Meier using the computer program SPSS for windows (version 6.0, SPSS Inc). Comparison of MMP positive and negative survival curves was performed using the log-rank test. Cox regression analysis was also performed to account for the effect of Dukes stage and age on survival.

EXAMPLE 2 - Colon and Ovarian Cancer

Colon cancers (n=64; age range of patients; 38-92; 38M 26F) which were submitted to the Department of Pathology, University of Aberdeen for diagnosis were used in this study. All the tumour samples had been fixed in 10% neutral buffered formalin and embedded in wax.

Histologically all the tumours were adenocarcinomas and pathological staging of the tumours was performed according to Dukes classification. There were 1 Dukes A, 38 Dukes B and 25 Dukes C. All the patients had survived at least one month following surgery and were followed-up for 46-52 months.

Positive immunoreactivity for MMP-1 was identified in 10 (16%) of the tumours while 54 (84%) tumours showed no expression of MMP-1. Five of the positive tumours were Dukes C, MMP-1 immunoreactivity was present in the cytoplasm of tumour cells and those tumours which were MMP-1 positive showed no significant intra-tumour heterogeneity of immunoreactivity. There were 8 (80%) deaths in the MMP-1 positive group while there were 27 (50%) deaths in the MMP-1 negative group. The survival of patients with MMP-1 positive tumours was significantly less compared with those patient whose tumours did not express MMP-1 ($p=0.006$, Figure). This remained significant after multi-variate analysis ($P=0.01$).

The immunohistochemistry method of Example 1 was also applied to 32 sections from ovarian cancer. The results are given in Figure 2. It will be apparent from Figure 2 that the cumulative survival rates in patients expressing MMP-9 was 60% at 40 months; wherein those patients with ovarian cancer but MMP-9 negative had an excellent prognosis in that all the samples were alive at 60 months.

Example 3 - Oesophageal Cancers

The immunohistochemistry method of example 1 was repeated using 90 sections from oesophageal cancers. The results shown in Figure 3 indicate that the survival of patients with MMP-1 positive tumours was significantly less compared with those tumours which did not produce MMP-1. In the same study, levels of MMP2, MMP9 and MMP11 did not significantly correlate with patient survival.

Example 4 - Therapeutic Systems

By way of exemplification of the treatment of poor prognosis colon cancer, the antibody from example 1 with specificity for MMP-1 was complexed with the radioactive label ^{131}I or ^{90}Y. Upon injection of the antibody-radiolabel complex into the patient, the antibody binds to MMP-1 associated with cells of the colon cancer and the accompanying radioactive emission irradiates and kills the cancer cells.

Alternatively by way of exemplification of the treatment of poor prognosis colon cancer, the antibody from example 1 with specificity for MMP-1 was complexed with a carboxypeptidase G2 enzyme. Upon injection of the antibody-carboxypeptidase complex into the patient, the antibody binds to MMP-1 associated with cells of the colon cancer. Following clearance of excess antibody not associated with the cancer, a pro-drug was injected into the patient which was converted

by the action of the carboxypeptidase to a cytotoxic drug which kills the cancer cells.

Degradation of the extracelluar matrix is an integral component in tumour invasion and spread, and MMPs are important in digesting different components of extracelluar connective tissue. Individual MMPs may be active at different stages of tumour spread. Initial tumour invasion depends on degradation of the basement membrane surrounding individual tumour cells while spread of established malignant tumours depends on degrading interstitial connective tissue. Therefore action of MMP-1 and MMP-9 which is the first step in digesting various components of interstitial connective tissue is possibly more important in facilitating the spread of established invasive tumours both locally and within distant organs. We have shown that expression of MMP-1 and MMP-9 in cancers are associated with a significantly poorer prognosis compared with those tumours which do not express MMP-1 or MMP-9. These results support the hypothesis that MMP-1 and MMP-9 expression is important for tumours to spread. MMP-1 degrades collagenase types I, II and III and increased collagenase activity towards type I and type III collagens is indicative of colorectal cancer. Although only 10 of the tumours expressed MMP-1, comparison of the survival of patients with MMP-1 positive tumours compared with patients with MMP-1 negative tumours showed that survival was significantly shorter.

A variety of histological and biological prognostic factors have been identified for colon cancer and generally these factors are not considered to be susceptible or modifiable by therapeutic intervention. In contrast expression of MMP-1 and MMP-9 are prognostic factors which are altered by therapeutic intervention as they are targets for MMP inhibitors.

Moreover, the monoclonal antibodies to MMP-1 and MMP-9 used in this study are reactive in formalin fixed wax embedded

sections and thus can be used in routine diagnostic histopathology practice to evaluate MMP status in colorectal or ovarian cancers and identify those patients who may benefit from anti-MMP therapy.

5

CLAIMS:

1. The method for the in vitro prognostic evaluation of cancer status from a sample which method comprises contacting said sample with a monoclonal or polyclonal antibody specific respectively for a selected matrix metalloproteinase and screening for a positive response.

2. A method according to claim 1 wherein the cancer is colorectal, oesophageal, ovarian or prostate cancer.

3. A method according to claim 1 or 2 wherein the selected matrix metalloproteinase is a collagenase or a gelatinase.

4. A method according to any preceding claim wherein the sample is a tissue or cell sample, a blood sample or a sample of a body product.

5. A method according to any preceding claim wherein the antibody is an antipeptide polyclonal or monoclonal antibody.

6. A method according to claim 5 wherein the polyclonal or monoclonal antibody is specific for a collagenase MMP-1 or for a gelatinase MMP-2 or MMP-9.

7. A method according to either of claims 5 or 6 wherein the antipeptide monoclonal antibody is reactive with amino acid residues 267 to 277 of the activated MMP-1 protein, or is reactive with amino acid residues 603 to 614 of the MMP-9 protein.

8. An antipeptide monoclonal antibody which is reactive with either amino acids 267 to 277 of the activated MMP-1 protein, or is reactive with amino acids 603 to 614 of the MMP-9 protein.

9. An antibody of claim 8 generated using a peptide synthesized with a cysteine residue included at the N-terminus of the peptide to couple the same to a carrier protein.

10. A therapeutic agent comprising an antipeptide monoclonal antibody to a collagenase or a gelatinase, or an agent derived from the antibody bound to a MMP inhibitory agent or a cytotoxic or imaging agent.

11. A therapeutic agent according to claim 10 wherein the agent derived from the antibody is a Fab^2 of Fab molecule, a scFv molecule or a humanised antibody molecule.

12. An agent according to claim 10 or 11 wherein the collagenase is MMP-1 and the antibody is reactive with amino acid residues 267 to 277 of the activated MMP-1 protein.

13. An agent according to claim 10 or 11 wherein the gelatinase is MMP-9 and the antibody is reactive with amino acid residues 603 to 614 of the MMP-9 protein.

14. An agent according to any of claims 10 to 13 wherein the MMP-1 or MMP-9 inhibitory agent has at least one effect selected from mimicking the protein residue which is cleaved from a pro-enzyme, reproducing the action of a TIMP, blocking the binding to the tumour cell surface or by inhibition of MMP catalytic sites.

15. An agent according to any of claims 10 to 14 comprising a pharmaceutically acceptable vehicle, said agent having been prepared for sale.

16. A prognostic kit comprising an antibody and including means for effecting the method of any one of claims 1 to 7.

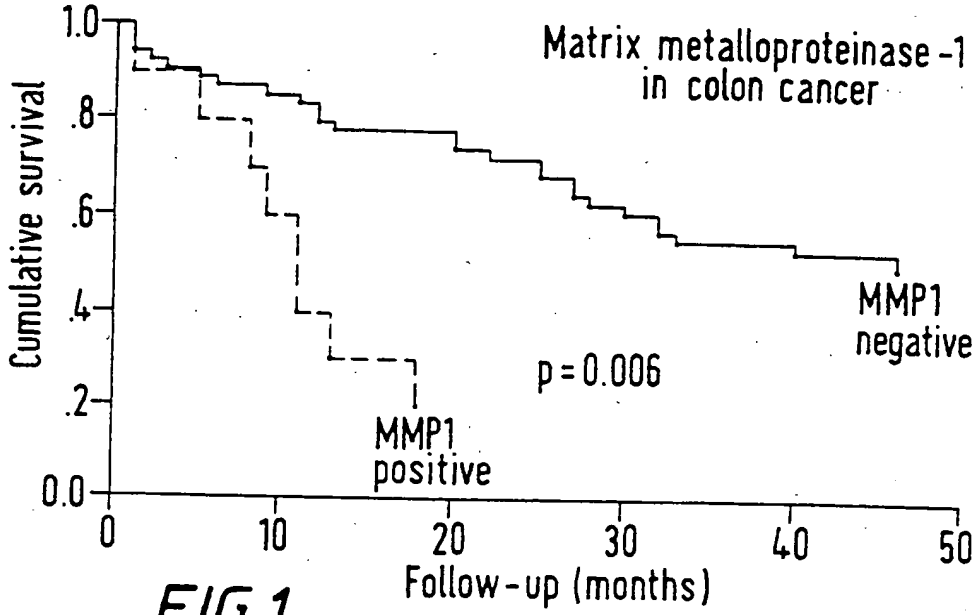
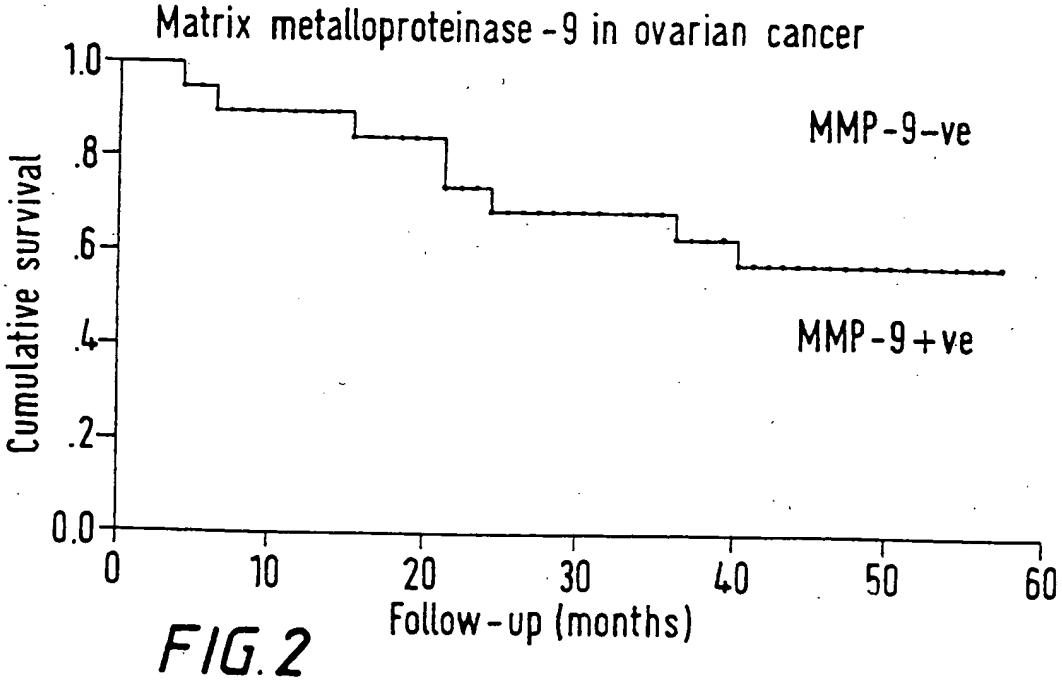

Matrix metalloproteinase-1
in colon cancer
Cumulative survival
1.0
.8
.6
.4
.2
0.0
MMP1
negative
p = 0.006
MMP1
positive
0
10
20
30
40
50
Follow-up (months)

FIG. 1


Matrix metalloproteinase-9 in ovarian cancer
Cumulative survival
1.0
.8
.6
.4
.2
0.0
MMP-9-ve
MMP-9+ve
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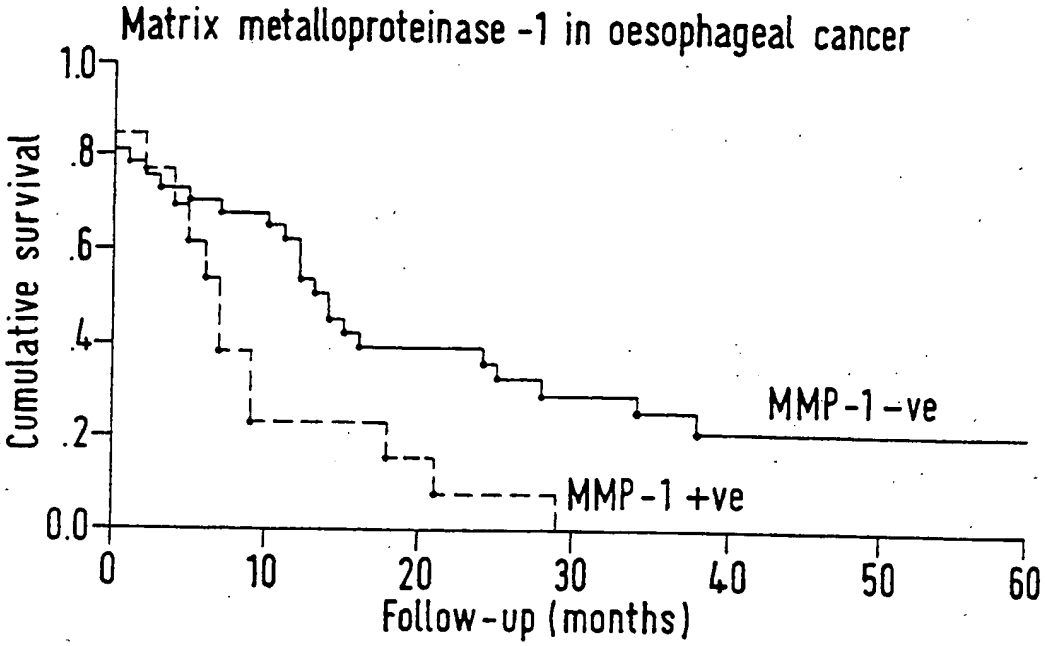
10
20
30
40
50
60
Follow-up (months)

FIG. 2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Cumulative survival
1.0
.8
.6
.4
.2
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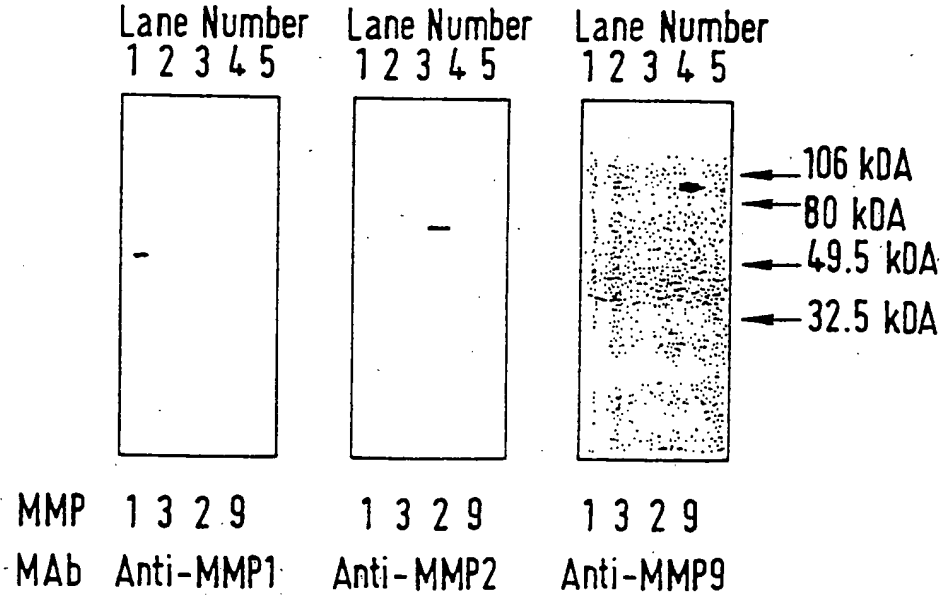
30
40
50
60
Follow-up (months)
MMP-1 -ve
MMP-1 +ve

FIG. 3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MAb Anti-MMP1
Anti-MMP2
Anti-MMP9

FIG. 4

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 96/01423

A. CLASSIFICATION OF SUBJECT MATTER
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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,93 20447 (THE RESEARCH FOUNDATION OF STATE UNIVERSITY OF NEW YORK) 14 October 1993 see the whole document	1-6,16
A	---	7-15
X	BRITISH JOURNAL OF CANCER, vol. 69, no. 1, 1994, CAMBRIDGE UK, pages 177-182, XP000609359 F.C. HAMDY ET AL.: "Matrix metalloproteinase 9 expression in primary human prostatic adenocarcinoma and benign prostatic hyperplasia." see page 177, column 2, line 16 - line 18 see page 178, column 2, line 21 - line 38 see page 181, column 2, line 16 - line 28	1-6,16
A	---	7-15
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

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Date of the actual completion of the international search	Date of mailing of the international search report
14 November 1996	22. 11. 96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Van Bohemen, C

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INTERNATIONAL SEARCH REPORT

Inter "onal Application No
PC1/GB 96/01423

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF IMMUNOLOGICAL METHODS, vol. 148, 1992, AMSTERDAM NL, pages 189-198, XP000609024 S. ZUCKER ET AL.: "Immunoassay of type IV collagenase / gelatinase (MMP-2) in human plasma" see page 197, column 2, line 13 - line 20; figure 2	1,3-6,16
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 96/01423

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9320447	14-10-93	US-A- 5324634	28-06-94
		AU-A- 3940793	08-11-93